

Establishment of an Animal Model to Evaluate the Biological Effects of Intramuscularly Embedded Depleted Uranium Fragments

Carl Andrew Castro

Kimberly A. Benson

Victor Bogo

Eric G. Daxon

John B. Hogan

Henry M. Jacocks

Michael R. Landauer

Sharon A. McBride

Christina W. Shehata

19960718 091

DTIC QUALITY INSPECTED 3

This and other AFRRRI publications are available to qualified users from the Defense Technical Information Center, Attention: OCP, 8725 John J. Kingman Road, Suite 0944, Fort Belvoir, VA 22060-6218; telephone (703) 767-8274. Others may contact the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161; telephone (703) 487-4650. AFRRRI publications are also available from university libraries and other libraries associated with the U.S. Government's Depository Library System.

Contents

Introduction	1
Methods	5
Subjects and Experimental Design	5
DU and Ta Pellets	5
Surgical Procedures for Pellet Implantation	5
Behavioral Measurements	6
Urinary Sampling and Collection Procedures	6
Determination of Urinary Uranium Levels	6
Results	7
Surgical Implantation	7
Locomotor Activity and Grip Strength	7
Body Weights, Food and Water Consumption, and Urinary Output	8
Urinary Uranium Levels	9
Discussion	11
Acknowledgements	11
References	13

Introduction

Natural uranium (U) consists of three isotopes: ^{238}U (99.276%), ^{235}U (0.718%), and ^{234}U (0.0056%). During the uranium enrichment process two isotopic mixtures are produced, "enriched uranium" and "depleted uranium" (DU) with different relative ratios of the three isotopes. Enriched uranium contains a higher percentage of the fissionable isotope ^{235}U and is used for nuclear reactor fuel and nuclear weapons. DU has a lower ^{235}U content. The DU used by the U.S. military for kinetic energy penetrators is alloyed with titanium (0.75% by weight) to increase its tensile strength and to retard oxidation. Current

U.S. antitank weapons contain DU penetrators, and most of the Abrams tanks are armored with DU. During Operation Desert Storm, DU munitions were fired by the Army and Air Force. Unfortunately, during this conflict, a number of U.S. military personnel were wounded by DU fragments (Daxon, 1993; Daxon and Musk, 1993; GAO Report, 1993). Many of these fragments were not removed because the surgical procedure would produce excessive tissue damage. Radiographs of injured soldiers show multiple embedded fragments ranging in size from 1 mm to over 5 mm in diameter (see figures 1 and

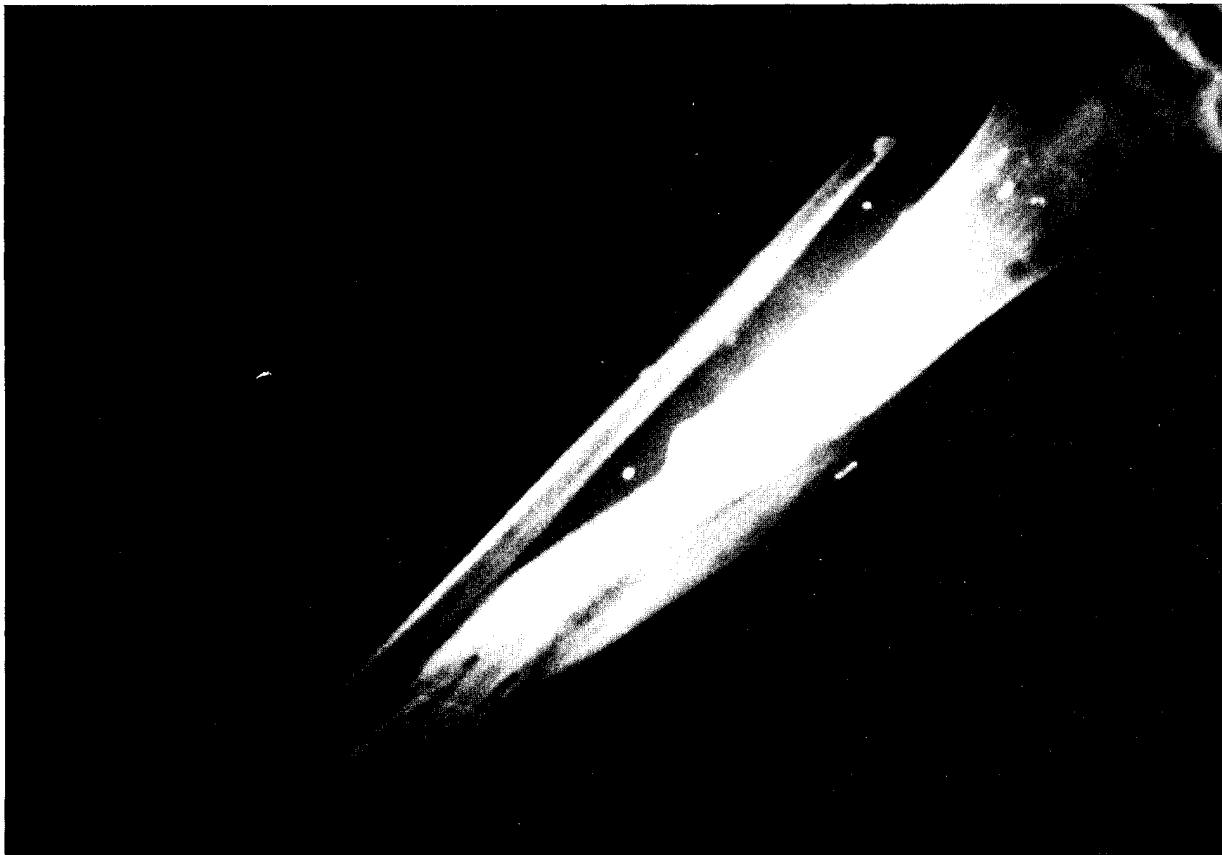


Fig. 1. Radiograph of the leg of a soldier wounded by a DU munition during the Persian Gulf War. This soldier also had DU fragments in the feet and knees of both legs.

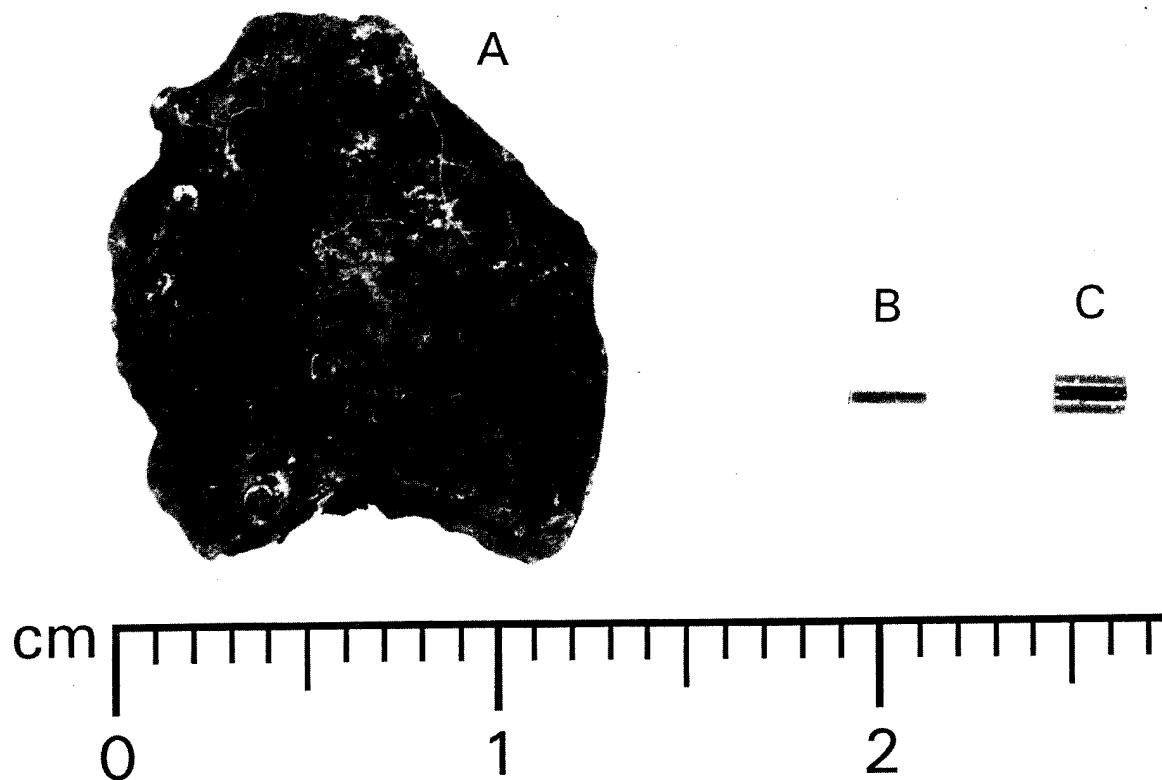


Fig. 2. (A) Photograph of an actual DU fragment removed from a soldier wounded during the Gulf War. (B) Photograph of a Ta pellet implanted in a rat. (C) Photograph of a DU pellet implanted in a rat.

2a). Indeed, fragments as large as 20 mm in diameter have been noted in other patients. Bioassays taken over a year after injury indicate that uranium was present at levels up to 30 μ g U/l urine, well in excess of natural background (U.S. Army Environmental Hygiene Agency Memorandum for Office of the Surgeon General, 1994).

Although the toxicity of embedded DU is unknown, numerous studies have addressed the consequences of inhalation, ingestion, and parenteral administration of other forms of uranium (Diamond, 1989; La Touche et al., 1987; Morrow et al., 1982; Ortega et al., 1989a, b; Wrenn et al., 1989). After uranium is absorbed, it circulates in the blood as the uranyl ion, forming uranium-carbonate and uranium-albumin complexes. As the uranium-carbonate complex passes through the kidney, it is filtered rapidly by the glomeruli where 60% to 80% of the absorbed uranium is excreted in the first 24 hours after acute exposure. The uranium that is not excreted is reabsorbed by the proximal tubules where it produces

significant toxic effects. Uranium also enters the bone, where it competes with calcium to form complexes with phosphate ions, thus becoming part of the bone matrix (Cabrini et al., 1984; Domingo et al., 1992; Guglielmotti et al., 1989; Neuman, 1950). This bone matrix then serves as both a long- and short-term storage site from which uranium is slowly released back into circulation (Kathren et al., 1989; Wrenn et al., 1985). The liver and muscle are other major sites of uranium deposition, with a possible long-term storage mechanism in the kidney (Kathren et al., 1989; Wrenn et al., 1985).

Acute morphological and biochemical changes of the kidney result from uranium exposure (Diamond, 1989; Kocher, 1989; Leggett, 1989; Neuman, 1950). Changes in the glomerular epithelial architecture (Kobayashi et al., 1984) and cellular necrosis in the proximal tubules near the corticomedullary junction of the kidney have been reported in experimental animals after acute uranium exposure (Brady et al., 1989; Haley et al., 1982; Haley, 1982). In addition,

polyuria, enzymuria, glucosuria, and increased excretion of amino acids have been demonstrated (Diamond, 1989; Diamond et al., 1989; Kocher, 1989; Zalups et al., 1988). Acute renal failure can indeed occur following exposure to high doses of uranium (Neuman, 1950; Ubios et al., 1994). Even acute environmental stressors such as restricted diets or changes in housing conditions have enhanced uranium toxicity significantly (Andrews and Bates, 1987; Damon et al., 1986).

Few studies have addressed the chronic toxicity of uranium, and the results available are conflicting (U.S. Department of Health and Human Services, 1990). Galibin and colleagues (1971) reported severe renal toxicity in rats that inhaled ammonium diuranate (1 or 8 mg/m³), a slightly soluble uranium compound, for 128 days. Urine protein and blood non-protein nitrogen were elevated. In the proximal tubules, there were sloughed dead cells and abnormal regenerating cells. Although the total number of tubules was reduced and the kidney exhibited an increased amount of connective tissue, all the animals recovered. In contrast, Leach and colleagues (1970; 1973) found no renal toxicity in rats repeatedly exposed to uranium dioxide dust (5 mg/m³) for a period of 12 months nor in dogs or monkeys exposed for 5 years. Yet uranium concentrations in the kidneys were as high as 1.1 µg U/g kidney wet weight in the rat, 8.3 µg U/g kidney weight in the dog, and 17.0 µg U/g kidney weight in the monkey. Uranium concentrations at these levels have been reported to cause acute renal toxicity (e.g., Kathren et al., 1989). Thus, the chronic effects of uranium exposure remain for the most part unresolved (Diamond, 1989).

The threshold concentration of kidney uranium levels in humans that result in kidney chemical toxicity is in dispute (Diamond, 1989; Kathren and Moore, 1986; Kocher, 1989; Stradling et al., 1988). While the Nuclear Regulatory Commission has set the level at 3.0 µg U/g kidney weight for renal damage in humans, there is evidence from both human and animal reports that this level could be considerably lower. For example, chronically exposed uranium mill workers, whose kidney uranium levels probably did not exceed 1 µg U/g kidney weight (Thun et al., 1985), showed mild renal dysfunction with increased urinary excretion of β₂-microglobulin and various amino acids. In rats exposed subchronically to low doses (cumulative dose: 0.66 or 1.32 mg/kg) of uranyl fluoride, kidney uranium levels as low as 0.7 to 1.4 µg U/g wet weight kidney produced cellular and tubular necrosis of the proximal tubule, proteinuria, and enzymuria (Diamond et al., 1989). These changes in rat renal function, however, were temporary, with complete recovery occurring within 35 days of exposure. These studies are important because they indicate that renal injury can occur at kidney uranium levels well below the 3.0 µg U/g limit.

Currently, no research into the direct toxic effects of embedded DU has been reported. The toxicity data that exist for low-level chronic uranium exposure used other routes of administration, and the results are contradictory. The uranium levels in humans that result in kidney toxicity are in dispute. For these various reasons, it is necessary to determine the health risks to the soldier resulting from long-term exposure to DU fragments. The goal of this pilot study was to establish an animal model that could be used in future research to investigate the biological effects of embedded DU.

Methods

Subjects and Experimental Design

Subjects were 12 naive Sprague-Dawley male rats (8-10 weeks old) obtained from Charles River Breeding Laboratories, Raleigh, N.C. On arrival, rats were quarantined and screened for diseases and were maintained in an AAALAC-accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23). Six rats were implanted with eight DU pellets (four in each biceps femoris muscle of the lateral thigh), and six rats were implanted with eight tantalum (Ta) pellets. Rats were individually housed in plastic Micro-Isolator cages with hardwood chips as bedding; during urine collection, rats were placed in metabolic cages. Commercial rodent chow and acidified water (pH 2.5, using concentrated HCl) were provided *ad libitum*. Rats were on a 12-hour light/dark cycle.

DU and Ta Pellets

DU pellets (1 mm in diameter x 2 mm in length) were obtained from Oak Ridge National Laboratories, Oak Ridge, Tenn. (see figure 2c). The cylindrical shape was chosen because it is the geometrical average of fragments left in soldiers wounded by conventional or DU munitions. The size of the pellets was based on two considerations. First, the total DU implanted was approximately 1% of the total biceps femoris muscle volume and did not seem to cause undue discomfort to the animal. Second, the surface area of 8 DU pellets of this size should result in detectable urinary uranium levels. DU pellets consisted of 99.25% DU and 0.75% titanium by weight. The uranium isotopes in DU were ^{238}U (99.75%), ^{235}U (0.25%), and trace amounts of ^{234}U . This is the same DU alloy used in U.S. military munitions.

Ta pellets (1 mm in diameter x 2 mm in length) were obtained from Alfa Products, Ward Hill, Mass., and served as the heavy metal control (see figure 2b). Ta

was selected because its density is similar to DU density, 16.6 g/cm^3 for Ta versus 18.8 g/cm^3 for DU (Radiological Health Handbook, 1970), it is relatively inert in a biological medium (Johansson et al., 1990), and it is commonly used in human orthopedic reconstructive surgery (Hockley et al., 1990).

Surgical Procedures for Pellet Implantation

Before implantation surgery, the DU and Ta pellets were cleaned by immersion in an industrial detergent, rinsed in absolute alcohol, sterilized by immersion in a 50% nitric acid solution for 3 minutes, rinsed with sterile water, and then placed in acetone to inhibit oxidation. These sterilization procedures completely remove the oxide formation from the surface of DU metal (Tonry, 1993), and the results of an abbreviated sterility test of 10 Ta pellets using either a thioglycollate medium or soybean-casein digest medium detected no microorganisms.

Rats were administered atropine (0.05 mg/kg i.m.) before being anesthetized. Anesthesia was induced with ketamine hydrochloride (50 mg/kg) in combination with xylazine hydrochloride (10 mg/kg) given i.p. in a 0.5-ml bolus, using a 25-gauge needle. These injections were administered intraperitoneally to prevent irritating the site of implantation. The surgical sites were then shaved and cleansed with Betadine. Four pellets were implanted approximately 15 mm apart in each biceps femoris muscle on the lateral side of each thigh. Using a scalpel blade, incisions were made through the skin and approximately 10 mm deep into the muscle mass. The proximal incisions were 10 mm distal to the iliac crest and were the implantation sites of the first pellets. Pellets were secured in place with absorbable sutures (Dexon 4-0) to prevent movement. Rats were closely monitored following surgery until they were ambulatory. A veterinarian or a veterinary technician examined the surgical sites for signs of inflammation, infection, and local DU toxicity daily for 2 weeks

following surgery and weekly thereafter throughout the study.

Behavioral Measurements

Locomotor activity and grip strength were assessed on days 3 and 5 before surgical implantation and on days 1, 3, 7, 14, 28, 60, and 120 after surgery. Locomotor activity was quantified using computerized Digiscan activity monitors (Omnitech Electronics, Columbus, Ohio). Each monitor used an array of infrared photodetectors spaced 2.5 cm apart to determine horizontal locomotor activity, which was expressed as total distance traveled. Activity was monitored for 1 h with measurements taken every 5 min (Landauer et al., 1988).

Immediately following locomotor activity testing, the strength of both hindlimb and forelimb grips of each animal was measured using a grip strength apparatus (San Diego Instruments, San Diego, Calif.). In this test, the animal was required to grip a rectangular wire mesh surface (12 x 7 cm) with its forepaws and was then gently pulled back along a platform until its grip was broken. The backward motion was continued until the animal's hindpaw gripped another rectangular wire mesh surface (12 x 10 cm). As with the forelimb grip, the animal was gently pulled back until the hindlimb grip was broken. Readings on three push-pull strain gauges were used to record the maximum strain required to break both forelimb and hindlimb grips. This behavioral test is used in many laboratories to assess muscular weakness (Haggerty, 1989; Meyer et al., 1979).

Urinary Sampling and Collection Procedures

Urine samples were collected following behavioral testing on days 1, 3, 7, 14, 28, 60, and 120 after surgery and analyzed for uranium levels. Sampling at these time points was necessary because signs of nephrotoxicity in laboratory animals exposed to low doses of uranium are frequently not detected until 3 to 5 days after exposure and may subside within 7

days (Diamond, 1989). Urine samples were collected from rats in individual metabolic cages (23.5 cm diameter x 12 cm high) where they had continuous access to food and water. Rats were acclimated to the metabolic cages for 5 days before the study began because naive rats exposed to these housing procedures have shown a stress-induced increase in uranium toxicity (Damon et al., 1986).

A 24-h urine sample was obtained from each rat, and the volume was recorded. In addition, each animal's body weight and food and water consumption were recorded. Care was taken to prevent contaminating the urine with food or feces. After collection, urine was filtered to remove any debris and stored in plastic containers at 4° C until analyzed. The metabolic cages were disinfected and decontaminated between each animal use. During animal-handling periods, overt signs of behavioral toxicity and the overall appearance of the rats were recorded.

Determination of Urinary Uranium Levels

Urinary uranium levels were determined by alpha spectrometric techniques (Martin Marietta Energy Systems, Inc., Oak Ridge, Tenn.). An aliquot of the sample was dissolved in nitric acid (HNO₃) and hydrogen peroxide (H₂O₂). The sample was then wet ashed, and the uranium coprecipitated with calcium oxalate. After dissolving the precipitate in HCl, the uranium was further separated by ion exchange chromatography. The uranium was then eluted from the column with a solution of dilute HCl to which titanous chloride had been added to reduce actinides that may have been in an elevated oxidation state. The final fraction of the eluate was treated first with ascorbic acid to reduce any iron and then with hydrofluoric acid. The uranium isotopes were next coprecipitated on neodymium fluoride. The neodymium was caught on a 0.1-μm filter, which was rinsed, dried, and then mounted on a planchet for alpha spectrometry. The minimum detectable activities (MDA) for uranium in urine using these procedures were 1.4 x 10⁻⁶ μg/l for ²³⁴U and 0.03 μg/l for ²³⁸U.

Results

Surgical Implantation

Two rats assigned to the DU group and one rat assigned to the Ta group did not survive implantation surgery. One of these rats expired during surgery, and the other two within 6 h after surgery. Necropsies indicated asphyxiation, suggesting that the animals received too much anesthetic. The other nine animals were alert and moving in the metabolic cages within 2 h after surgery. Figure 3 is a radiograph of the left rear leg of a rat implanted with four DU pellets; the right rear leg was also implanted with four DU pellets. The cylindrical shape and size of the pellets are similar to DU fragments observed in wounded soldiers (figure 1).

Locomotor Activity and Grip Strength

The locomotor activity of rats implanted with DU pellets was not significantly different from the activity of rats implanted with Ta, $p > 0.05$ (figure 4).

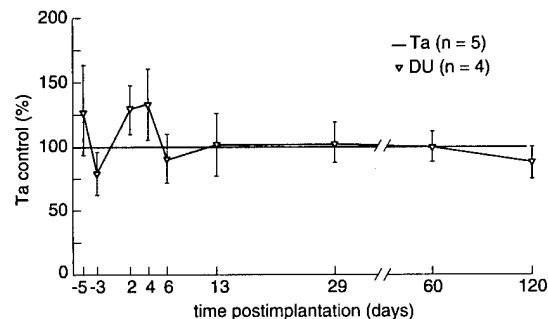


Fig. 4. Locomotor activity of rats surgically implanted with DU pellets expressed as percent of Ta control. Vertical bars represent the SEM (standard error of the mean).



Fig. 3. Radiograph of the left rear leg of a rat surgically implanted with four DU pellets (1 mm in diameter x 2 mm in length).

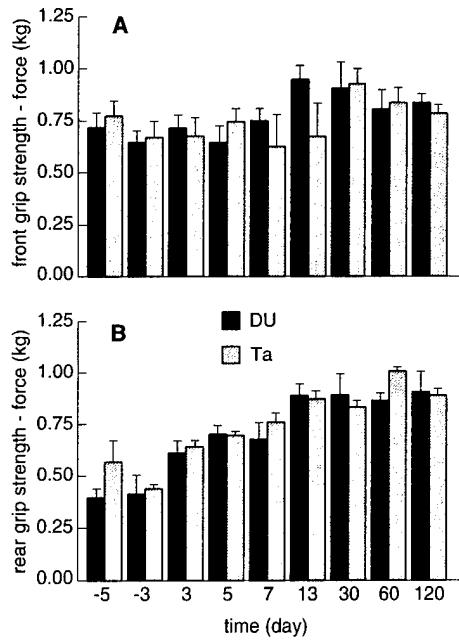


Fig. 5. (A) Forelimb grip strength of rats before and after receiving DU or Ta implants. Vertical bars represent the SEM. (B) Hindlimb grip strength of rats before and after receiving DU or Ta implants. Vertical bars represent the SEM.

Similarly, neither the forelimb nor the hindlimb grip strength of the two groups was different, $p > 0.05$ (figures 5a and 5b).

Body Weights, Food and Water Consumption, and Urinary Output

The body weights of the rats embedded with DU pellets were not different than the body weights of rats embedded with Ta, $p > 0.05$ (figure 6). In fact,

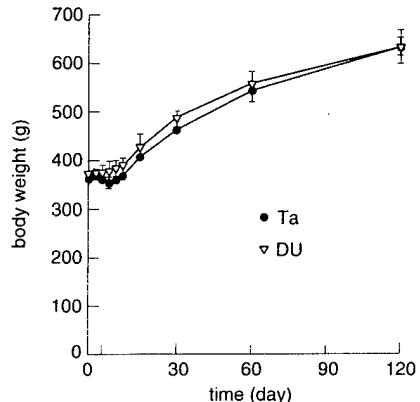


Fig. 6. Body weights of rats before and after receiving DU or Ta implants. Vertical bars represent the SEM.

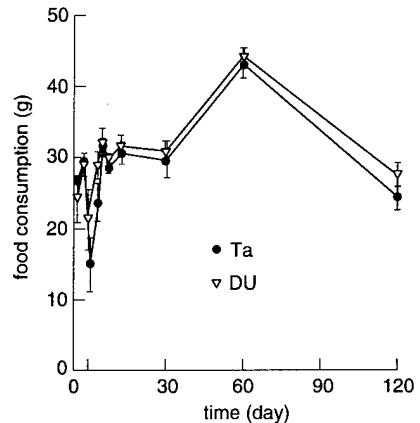


Fig. 7. Food consumption of rats before and after receiving DU or Ta implants. Vertical bars represent the SEM.

the body weights in both groups remained relatively stable for the first week following surgery and, as expected, increased throughout the study as observed in normal rats.

The food and water consumption for the DU- and Ta-implanted rats did not differ, $p > 0.05$ (figures 7 and 8). There was, however, a trend toward a decrease in water consumption for the Ta group and an increase in water consumption for the DU group.

There was a significant difference in the volume of urinary output between the DU and Ta groups. On the day of surgery, urine output for the Ta group decreased but did not change for the DU group, p

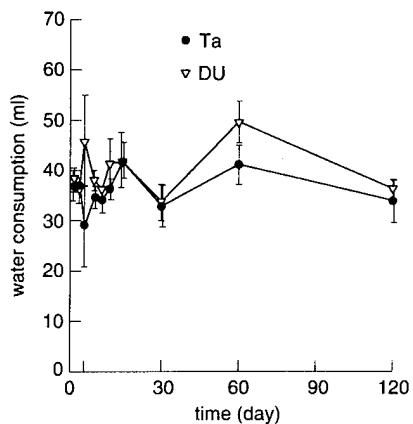


Fig. 8. Water consumption of rats before and after receiving DU or Ta implants. Vertical bars represent the SEM.

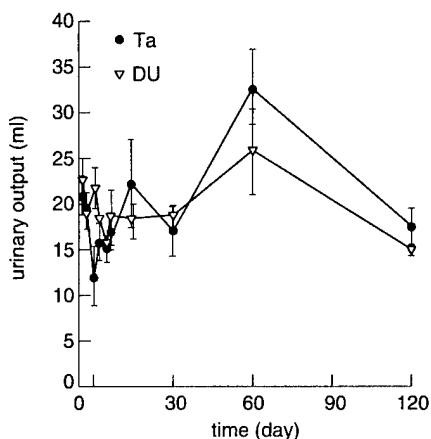


Fig. 9. Urinary output of rats before and after receiving DU or Ta implants. Vertical bars represent the SEM.

<0.05 (figure 9). This decrease in the urinary output for the Ta group, however, was temporary and returned to baseline levels by day 3 after surgery.

Urinary Uranium Levels

Figure 10 illustrates mean uranium levels in the urine of DU-implanted animals and the pooled value of the uranium analysis for Ta-implanted animals after implantation surgery. Figure 11 provides the individual urinary uranium levels of the four DU-implanted rats. As expected, only background levels of uranium were detected in the Ta control group. In contrast, significant levels of uranium were detected within 24 h of DU implantation (mean = 28.69 ± 10.00 ,

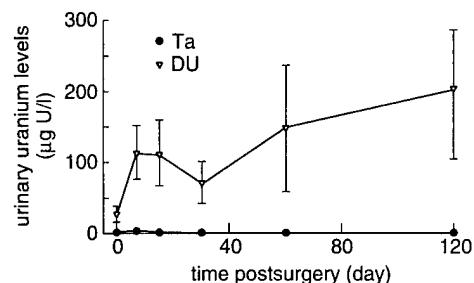


Fig. 10. Time course of uranium levels detected in the urine of rats implanted with either DU or Ta. Uranium concentration detected in the Ta group is at background levels. Vertical bars for the DU group ($N = 4$) represent the SEM. Urine for the Ta-implanted animals was pooled for uranium analyses.

range = 14.21 to 56.99 $\mu\text{g U/l}$). By day 7 following surgery, uranium levels had increased nearly four-fold (mean = 111.86 ± 41.05 , range = 56.38 to 233.91 $\mu\text{g U/l}$) and remained elevated at day 120 (mean = 204.56 ± 99.73 , range = 35.01 to 458.53 $\mu\text{g U/l}$).

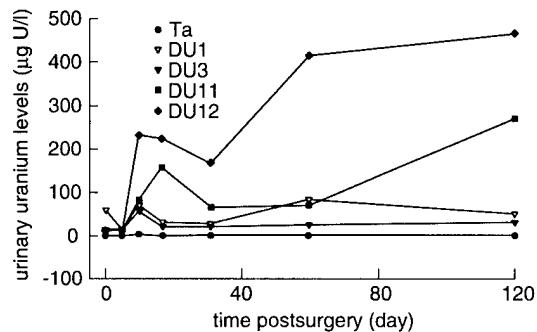


Fig. 11. Individual time courses of uranium levels detected in the urine of each rat implanted with DU. Data on Ta time courses are the same as in figure 10.

Discussion

The purpose of this study was to develop an animal model that could be used in future research to determine the health risks associated with DU fragment injuries. It was especially important to establish procedures in which DU exposure would produce urinary uranium levels comparable to those observed in soldiers wounded by DU munitions during the Persian Gulf War. Measured by these criteria, this initial study was successful. The average urinary uranium level in the rat 24 h after DU implantation was 28.69 $\mu\text{g U/l}$. This value is very close to the urinary level of 30 $\mu\text{g U/l}$ reported for soldiers wounded during the Persian Gulf War and assayed 1 year after injury. Unfortunately, no bioassays were taken of any of the soldiers within the first year after DU injury so no direct time course comparisons can be made.

It should be emphasized that the urinary uranium levels in the rat did not reach asymptote until day 7 following DU implantation surgery and remained elevated throughout the study (figure 10). Although the data are preliminary, this finding has clinical significance because it indicates that soldiers with suspected DU fragment wounds should be monitored for uranium exposure for at least the first week after injury and perhaps even longer. Certainly a complete pharmacokinetic study should be conducted to definitively address this patient-monitoring issue (Daxon, 1993).

Although numerous studies have assessed the toxic effects of other forms of uranium exposure (Diamond, 1989, and Kocher, 1989, for the latest reviews of the literature), this is the first study that assessed the effects of intramuscularly embedded DU. The rat proved to be an excellent animal model for this

purpose. It tolerated the surgical procedures for pellet implantation relatively well, as measured by both locomotor activity and grip strength (figures 4 and 5), both indices of quality of life for humans. Further, the lateral thigh muscle of the adult rat is large enough to implant at least four pellets (1.0 mm diameter x 2 mm length) into each leg (figure 3), with the possibility of as many as ten pellets. Moreover, the rat's lifespan of more than 18 months enables it to be used in chronic toxicity studies (Brady et al., 1989; Lang and White, 1994; Lumley et al., 1992; Lumley and Walker, 1986; Monro, 1993; Nohynek et al., 1993; Rao et al., 1990).

In conclusion, this study was successful in developing a rodent model that can be used to evaluate the biological effects of intramuscularly embedded DU fragments. However, the potential short-term and long-term health risks associated with DU exposure remain to be investigated. Certainly the behavioral, physiological, biochemical, and histological consequences of embedded DU are research areas of immediate concern. Equally important is identification of the health risks to the fetus exposed *in utero* to DU from fragments embedded in the mother before pregnancy (Angleton et al., 1988; Bosque et al., 1993; Domingo et al., 1988a, b, c; Paternain et al., 1989). This latter research area is especially significant considering that the placenta does not prevent cross-placental transfer of uranium (Durbin and Wrenn, 1976; Sikov and Mahlum, 1968). Moreover, fetal toxicity often occurs in the absence of maternal toxicity (e.g., Price et al., 1985). Regardless of the research strategy adopted, a coordinated interdisciplinary health hazard assessment is required to identify the potential medical risks that DU poses to our soldiers wounded by this unconventional munition.

Acknowledgements

We thank Ms. Elizabeth L. Wampler for radiation safety advice, Major Rebecca A. Cockman-Thomas for performing surgical implantations, Dr. G. David Ledney and Dr. Thomas B. Elliott for conducting and interpreting pellet sterility tests, Mr. William E. Jackson III for statistical advice, and Ms. Modeste E. Greenville and Ms. Carolyn Wooden for publication assistance.

References

Andrews PM, Bates SB (1987) Effects of dietary protein on uranyl-nitrate-induced acute renal failure. *Nephron* 45:296-301

Angleton GM, Benjamin SA, Lee AC (1988) Health effects of low-level irradiation during development: Experimental design and prenatal and early neonatal mortality in beagles exposed to ^{60}Co gamma rays. *Radiation Research* 115:70-83

Bosque MA, Domingo JL, Llobet JM, Corbella J (1993) Embryotoxicity and teratogenicity of uranium in mice following subcutaneous administration of uranyl acetate. *Biological Trace Element Research* 36:109-118

Brady HR, Kone BC, Brenner RM, Gullans SR (1989) Early effects of uranyl nitrate on respiration and K^+ transport. *Kidney International* 36: 27-34

Cabrini RL, Guglielmotti MB, Ubios AM (1984) Prevention of the toxic effect of uranium on bone formation by tetracycline. *Acta Odontologica Latinoamericana* 1:61-63

Damon EG, Eidson AF, Hobbs CH, Hanff FF (1986) Effects of acclimation to caging on nephric response of rats to uranium. *Laboratory Animal Science* 36:24-27

Daxon EG (1993) Protocol for monitoring Gulf War veterans with imbedded fragments of depleted uranium. AFRRRI Technical Report TR93-2, Armed Forces Radiobiology Research Institute, Bethesda, MD

Daxon EG, Musk JH (1993) Assessment of the risks from imbedded fragments of depleted uranium. AFRRRI Technical Report TR93-1, Armed Forces Radiobiology Research Institute, Bethesda, MD

Diamond GL (1989) Biological consequences of exposure to soluble forms of natural uranium. *Radiation Protection Dosimetry* 26:23-33

Diamond GL, Morrow PE, Panner BJ, Gelein RM, Baggs RB (1989) Reversible uranyl fluoride nephrotoxicity in the Long-Evans rat. *Fundamental and Applied Toxicology* 13:65-78

Domingo, JL, Ortega A, Llobet JM, Paternain JL, Corbella J (1989a) The effects of repeated parenteral administration of chelating agents on the distribution and excretion of uranium. *Research Communications in Chemical Pathology and Pharmacology* 64:161-164

Domingo JL, Ortega A, Paternain JL, Jacinto C (1989b) Evaluation of the perinatal and postnatal effects of uranium in mice upon oral administration. *Archives of Environmental Health* 44:395-398

Domingo JL, Paternain JL, Llobet JM, Corbella J. (1989c) The developmental toxicity of uranium in mice. *Toxicology* 55:143-152

Domingo JL, Colomina MT, Llobet JM, Jones MM, Singh PK (1992) The action of chelating agents in experimental uranium intoxication in mice: Variations with structure and time of administrations. *Fundamental and Applied Toxicology* 19: 350-357

Durbin PW, Wrenn ME (1976) Metabolism and effects of uranium in animals. In: Conference on Occupational Health Experience with Uranium. U.S. Energy Research and Development Administration, WA 470 C75c, Washington, D.C., 68-99

Galibin GP, Vlasov PA, Fedoseyeva LA (1971) Remote aftereffects of killing rats using ammonium diurinate. In: *Otdalennye Posledstviya*

Luchevykh Porazhenii, Moskalev UI (ed), pp 197-206, Atomizdat, Moscow; English translation, AEC-TR-7387

GAO Report (1993) Army not adequately prepared to deal with depleted uranium contamination. GAO/NISAID-93-90

Guglielmotti MB, Ubios AM, Larumbe J, Cabrini RL (1989) Tetracycline in uranyl nitrate intoxication: Its action on renal damage and U retention in bone. *Health Physics* 57:403-405

Haggerty GC (1989) Development of tier I neurobehavioral testing capabilities for incorporation into pivotal rodent safety assessment studies. *Journal of the American College of Toxicology* 8:53-69

Haley DP (1982) Morphologic changes in uranyl nitrate-induced acute renal failure in saline- and water-drinking rats. *Laboratory Investigation* 46: 196-207

Haley DP, Bulger RE, Dobyan DC (1982) The long-term effects of uranyl nitrate on the structure and function of the rat kidney. *Virchows Archives [Cell Pathology]* 41(1-2):181-192

Hockley AD, Goldin JH, Wake MJC, Iqbal J (1990) Skull repair in children. *Pediatric Neurosurgery* 16:271-275

Johansson CB, Hansson HA, Albrektsson T (1990) Qualitative interfacial study between bone and tantalum, niobium or commercially pure titanium. *Biomaterials* 11:277-280

Kathren RL, McInroy JF, Moore RH, Dietert SE (1989) Uranium in the tissues of an occupationally exposed individual. *Health Physics* 57:17-21

Kathren RL, Moore RH (1986) Acute accidental inhalation of U: A 38-year follow-up. *Health Physics* 51:609-619

Kobayashi S, Nagase M, Honda N, Hishida A (1984) Glomerular alterations in uranyl acetate-induced acute renal failure in rabbits. *Kidney International* 26: 808-815

Kocher DC (1989) Relationship between kidney burden and radiation dose from chronic ingestion of U: Implications for radiation standards for the public. *Health Physics* 57:9-15

Landauer MR, Davis HD, Dominitz JA, Weiss JF (1988) Long-term effects of radioprotector WR-2721 on locomotor activity and body weight of mice following exposure to ionizing radiation. *Toxicology* 49:315-323

Lang PL, White WJ (1994) Growth, development, and survival of the Crl:CD(SD)BR stock and CDF(F344)/CrlBR strain. *Pathobiology of the Aging Rat* 2:587-608

La Touche YD, Willis DL, Dawydiak OI (1987) Absorption and biokinetics of U in rats following oral administration of uranyl nitrate solution. *Health Physics* 53:147-162

Leach LJ, Maynard EA, Hodge HC, Scott JK, Yuile CL, Sylvester GE, Wilson HB (1970) A five year inhalation study with uranium dioxide (UO_2) dust. I. Retention and biologic effect in the monkey, dog, and rat. *Health Physics* 18:599-612

Leach LJ, Yuile CL, Hodge HC (1973) A five year inhalation study with uranium dioxide (UO_2) dust. II. Postexposure retention and biologic effect in the monkey, dog, and rat. *Health Physics* 25:239-258

Leggett RW (1989) The behavior and chemical toxicity of U in the kidney: A reassessment. *Health Physics* 57:365-383

Lumley CE, Parkinson C, Walker SR (1992) An international appraisal of the minimum duration of chronic animal toxicity studies. *Human and Experimental Toxicology* 11:155-162

Lumley CE, Walker SR (1986) A critical appraisal of the duration of chronic animal toxicity studies. *Regulatory Toxicology and Pharmacology* 6:66-72

U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD, Memorandum for Office of the Surgeon General, PSP, Subject: Results of analyzing urine bioassay specimens for uranium (Interim Report), 20 April 1994

Meyer OA, Tilson HA, Byrd WC, Riley MT (1979) A method for the routine assessment of forelimb and hindlimb grip strength of rats and mice. *Neurobehavioral Toxicology* 1:233-236

Monro A (1993) How useful are chronic (life-span) toxicology studies in rodents in identifying pharmaceuticals that pose a carcinogenic risk to humans? *Adverse Drug Reactions and Toxicology Reviews* 12(1):5-34

Morrow P, Gelein R, Beiter H, Scott J, Picano J, Yuile C (1982) Inhalation and intravenous studies of UF6/UO2F in dogs. *Health Physics* 43: 859-873

Neuman WF (1950) Urinary uranium as a measure of exposure hazard. *Industrial Medicine and Surgery* 19:185-191

Nohynek GJ, Longeart L, Geffray B, Provost JP, Lodola A (1993) Fat, frail and dying young: Survival, body weight and pathology of the Charles River Sprague-Dawley-derived rat prior to and since the introduction of the VAF^R variant in 1988. *Human Experimental Toxicology* 12:87-98

Ortega A, Domingo JL, Gomez M, Corbella J (1989a) Treatment of experimental acute uranium poisoning by chelating agents. *Pharmacology and Toxicology* 64:247-251

Ortega A, Domingo JL, Llobet JM, Thomas JM, Paternain JL (1989b) Evaluation of the oral toxicity of uranium in a 4-week drinking study in rats. *Bulletin of Environmental Contamination and Toxicology* 42:935-941

Paternain JL, Domingo JL, Ortega A, Llobet JM (1989) The effects of uranium on reproduction, gestation, and postnatal survival in mice. *Eco-toxicology and Environmental Safety* 17:291-296

Price CJ, Kimmel CA, Tyl RW, Marr MC (1985) The developmental toxicity of ethylene glycol in rats and mice. *Toxicology and Applied Pharmacology* 81:113-127

Radiological Health Handbook, U.S. Department of Health, Education, and Welfare, Public Health Service, Ed., Bureau of Radiological Health and Training, Institute of Environmental Control Administration, p. 65, 1970

Rao GN, Haseman JK, Grumbein S, Crawford DD, Eustis SL (1990) Growth, body weight, survival, and tumor trends in F344/N rats during an eleven-year period. *Toxicologic Pathology* 18:61-70

Sikov MR, Mahlum DD (1968) Cross-placental transfer of selected actinides in the rat. *Health Physics* 14:205-208

Stradling GN, Stather JW, Gray SA, Moody JC, Hodgson A, Cooke N (1988) The metabolism of ceramic and non-ceramic forms of uranium dioxide after deposition in the rat lung. *Human Toxicology* 7(2):133-139

Thun MJ, Baker DB, Steenland K, Smith AB, Halperin W, Berl T (1985) Renal toxicity of uranium mill workers. *Scandinavian Journal of Work, Environment and Health* 11:83-90

Tony LL (1993) Solubility of depleted uranium fragments within simulated lung fluid. Unpublished Dissertation, Boston University

Ubios AM, Braun EM, Cabrini RL (1994) Lethality due to uranium poisoning is prevented by ethane-1-hydroxy-1,1-biphosphonate (EHBP). *Health Physics* 66:540-544

U.S. Department of Health and Human Services (1990) Toxicological profile for uranium, TP-90-29. U.S. Government Printing Office, Washington, DC

Wrenn ME, Durbin PW, Howard B, Lipszten J, Rundo J, Still ET, Willis DL (1985) Metabolism of ingested U and Ra. *Health Physics* 48:601-633

Wrenn ME, Lipszten J, Bertelli L (1989) Pharmacokinetic models relevant to toxicity and metabolism for uranium in humans and animals. *Radiation Protection Dosimetry* 26:243-248

Zalups RK, Gelein RM, Morrow PE, Diamond GL (1988) Nephrotoxicity of uranyl fluoride in uninephrectomized and sham-operated rats. *Toxicology and Applied Pharmacology* 94:11-22

DISTRIBUTION LIST

DEPARTMENT OF DEFENSE

ARMED FORCES RADIobiology RESEARCH INSTITUTE
ATTN: PUBLICATIONS BRANCH
ATTN: LIBRARY

ARMY/AIR FORCE JOINT MEDICAL LIBRARY
ATTN: DASG-AAFJML

ASSISTANT TO THE SECRETARY OF DEFENSE
ATTN: AE
ATTN: HA(IA)

DEFENSE NUCLEAR AGENCY
ATTN: TITL
ATTN: DDIR
ATTN: RAEM
ATTN: MID

DEFENSE TECHNICAL INFORMATION CENTER
ATTN: ACQUISITION
ATTN: ADMINISTRATOR

FIELD COMMAND DEFENSE NUCLEAR AGENCY
ATTN: DASIAc
ATTN: FCIEO

INTERSERVICE NUCLEAR WEAPONS SCHOOL
ATTN: DIRECTOR

LAWRENCE LIVERMORE NATIONAL LABORATORY
ATTN: LIBRARY

UNDER SECRETARY OF DEFENSE (ACQUISITION)
ATTN: OUSD(A)/R&E

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
ATTN: LIBRARY

DEPARTMENT OF THE ARMY

HARRY DIAMOND LABORATORIES
ATTN: SLCM-SE

OFFICE OF THE SURGEON GENERAL
ATTN: MEDDH-N

U.S. ARMY AEROMEDICAL RESEARCH LABORATORY
ATTN: SCIENCE SUPPORT CENTER

U.S. ARMY CHEMICAL RESEARCH, DEVELOPMENT, & ENGINEERING CENTER
ATTN: SMCCR-RST

U.S. ARMY INSTITUTE OF SURGICAL RESEARCH
ATTN: COMMANDER

U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL
ATTN: MCCS-FCM

U.S. ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
ATTN: COMMANDER

U.S. ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE
ATTN: MCMR-UV-R

U.S. ARMY NUCLEAR AND CHEMICAL AGENCY
ATTN: MONA-NU

U.S. ARMY RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE
ATTN: DIRECTOR OF RESEARCH

U.S. ARMY RESEARCH LABORATORY
ATTN: DIRECTOR

WALTER REED ARMY INSTITUTE OF RESEARCH
ATTN: DIVISION OF EXPERIMENTAL THERAPEUTICS

DEPARTMENT OF THE NAVY

BUREAU OF MEDICINE & SURGERY
ATTN: CHIEF

NAVAL AEROSPACE MEDICAL RESEARCH LABORATORY
ATTN: COMMANDING OFFICER

NAVAL MEDICAL RESEARCH AND DEVELOPMENT COMMAND
ATTN: CODE 42

NAVAL MEDICAL RESEARCH INSTITUTE
ATTN: LIBRARY

NAVAL RESEARCH LABORATORY
ATTN: LIBRARY

OFFICE OF NAVAL RESEARCH
ATTN: BIOLOGICAL & BIOMEDICAL S&T

DEPARTMENT OF THE AIR FORCE

BROOKS AIR FORCE BASE
ATTN: AL/OEBZ
ATTN: OEHL/RZ
ATTN: USAFSAM/RZB

OFFICE OF AEROSPACE STUDIES
ATTN: OAS/XRS

OFFICE OF THE SURGEON GENERAL
ATTN: HQ AFMOA/SGPT
ATTN: HQ USAF/SGES

U.S. AIR FORCE ACADEMY
ATTN: HQ USAFA/DFBL

U.S. AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
ATTN: DIRECTOR OF CHEMISTRY & LIFE SCIENCES

OTHER FEDERAL GOVERNMENT

ARGONNE NATIONAL LABORATORY
ATTN: ACQUISITIONS

BROOKHAVEN NATIONAL LABORATORY
ATTN: RESEARCH LIBRARY, REPORTS SECTION

CENTER FOR DEVICES AND RADIological HEALTH
ATTN: DIRECTOR

GOVERNMENT PRINTING OFFICE
ATTN: DEPOSITORY ADMINISTRATION BRANCH
ATTN: CONSIGNMENT BRANCH

LIBRARY OF CONGRESS
ATTN: UNIT X

LOS ALAMOS NATIONAL LABORATORY
ATTN: REPORT LIBRARY

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
ATTN: RADLAB

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
GODDARD SPACE FLIGHT CENTER
ATTN: LIBRARY

NATIONAL CANCER INSTITUTE
ATTN: RADIATION RESEARCH PROGRAM

NATIONAL DEFENSE UNIVERSITY
ATTN: LIBRARY

NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
ATTN: IONIZING RADIATION DIVISION

U.S. DEPARTMENT OF ENERGY
ATTN: LIBRARY

U.S. FOOD AND DRUG ADMINISTRATION
ATTN: WINCHESTER ENGINEERING AND
ANALYTICAL CENTER

U.S. NUCLEAR REGULATORY COMMISSION
ATTN: LIBRARY

RESEARCH AND OTHER ORGANIZATIONS

AUSTRALIAN DEFENCE FORCE
ATTN: SURGEON GENERAL

AUTRE, INC.
ATTN: PRESIDENT

BRITISH LIBRARY
ATTN: ACQUISITIONS UNIT

CENTRE DE RECHERCHES DU SERVICE DE SANTE DES ARMEES
ATTN: DIRECTOR

FEDERAL ARMED FORCES DEFENSE SCIENCE AGENCY FOR
NBC PROTECTION
ATTN: LIBRARY

INHALATION TOXICOLOGY RESEARCH INSTITUTE
ATTN: LIBRARY

INSTITUTE OF RADIobiology, ARMED FORCES
MEDICAL ACADEMY
ATTN: DIRECTOR

OAK RIDGE ASSOCIATED UNIVERSITIES
ATTN: MEDICAL LIBRARY

RESEARCH CENTER OF SPACECRAFT RADIATION SAFETY
ATTN: DIRECTOR

RUTGERS UNIVERSITY
ATTN: LIBRARY OF SCIENCE AND MEDICINE

UNIVERSITY OF CALIFORNIA
ATTN: DIRECTOR, INSTITUTE OF TOXICOLOGY &
ENVIRONMENTAL HEALTH
ATTN: LIBRARY, LAWRENCE BERKELEY LABORATORY

UNIVERSITY OF CINCINNATI
ATTN: UNIVERSITY HOSPITAL, RADIOISOTOPE
LABORATORY

XAVIER UNIVERSITY OF LOUISIANA
ATTN: COLLEGE OF PHARMACY

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED	
	July 1996	Technical Report	
4. TITLE AND SUBTITLE		5. FUNDING NUMBERS	
Establishment of an Animal Model to Evaluate the Biological Effects of Intramuscularly Embedded Depleted Uranium Fragments		PE: NWED QAXM	
6. AUTHOR(S)		8. PERFORMING ORGANIZATION REPORT NUMBER	
Castro CA, Benson KA, Bogo V, Daxon EG, Hogan JB, Jacocks HM, Landauer MR, McBride SA, Shehata CW		TR96-3	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)		10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
Armed Forces Radiobiology Research Institute 8901 Wisconsin Avenue Bethesda, MD 20889-5603			
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		11. SUPPLEMENTARY NOTES	
Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20814-4799			
12a. DISTRIBUTION/AVAILABILITY STATEMENT		12b. DISTRIBUTION CODE	
Approved for public release; distribution unlimited.			
13. ABSTRACT (Maximum 200 words)			
<p>During the Persian Gulf War, 36 U.S. soldiers were wounded by depleted uranium (DU) munitions. Based on medical guidelines for conventional shrapnel injuries (nonradioactive), many DU fragments were left in soldiers. Unfortunately, health risks associated with embedded DU were unknown, and an animal model to investigate this did not exist. The purpose of this study was to develop an animal model to examine the health risks associated with DU shrapnel injuries. Twelve rats were surgically implanted intramuscularly with 8 DU pellets (1 mm in diameter x 2 mm in length) or 8 chemically inert tantalum (Ta) pellets of similar size. Urinary uranium levels were measured on days 1, 3, 7, 14, 28, 60, and 120 after implantation of DU pellets. Physiological and behavioral parameters, including locomotor activity, forelimb and hindlimb grip strength, food and water consumption, and urinary output, were measured 5 and 3 days before surgery and on days 1, 3, 7, 14, 28, 60, and 120 after surgery. Urinary uranium levels for Ta-implanted rats remained at background levels. In contrast, the average urinary uranium level for DU-implanted rats was significantly elevated on day 1 (28.69 µg U/l) after implantation and remained elevated until day 120 (204.56 µg U/l). There was no significant difference between DU- and Ta-implanted rats in any behavioral or physiological measures. Results indicate that the rat is an appropriate animal model for evaluating biological effects of embedded DU fragments.</p>			
14. SUBJECT TERMS		15. NUMBER OF PAGES 21	
		16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL

SECURITY CLASSIFICATION OF THIS PAGE

CLASSIFIED BY:

DECLASSIFY ON: